Evaluation of Dharmacon™ Accell™ siRNA delivery into spheroids and hiPSCs

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Introduction

Researchers are using more complex tissue models to study a variety of diseases, including hiPSC-differentiated cells and 3D spheroids. 3D tumor spheroid models offer numerous advantages over conventional two-dimensional cell culture, which include the development of a heterogeneous architecture resulting in apical-basal polarity, and an internal gradient of nutrient, oxygen, and signaling factors. Additionally, 3D models provide the ability to study the biochemical and mechanical interaction of cells with extracellular matrix proteins. Therefore, tumor spheroids resemble tumors grown in vivo more closely than 2D monolayer culture. Given the advantages of 3D culture, we sought to determine if standard spheroid culture models were susceptible to transfection-reagent free siRNA delivery using Dharmacon Accell siRNA. We optimized culture conditions for three commonly used spheroid models: seeding cells into ultralow attachment (ULA) plates, into Matrigel™, and into GrowDex®. We tested various serum conditions, after recent literature reported that complexing siRNA in high serum can assist with siRNA delivery into spheroids. Furthermore, we tested the efficiency of multiplexing Accell siRNA in HCT-116 spheroids by targeting two genes simultaneously. Using these tools, we aim to guide researchers in this field, who may be seeking guidance for siRNA delivery in complex cell culture models. In the future we plan to expand these studies to hiPSC-derived organoids, such as brain and kidney. Developing robust protocols for siRNA delivery in complex cell models will enable researchers to generate reproducible results and will potentially advance the field of 3D cell culture.

Transfection-free RNAi with Accell siRNA into complex models

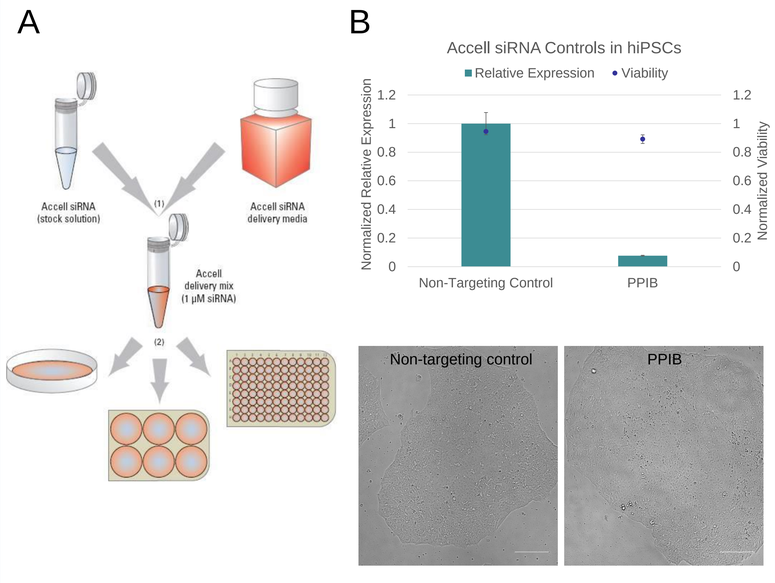


Fig 1. Transfection-free Accell siRNA reagent for complex models. A) Schematic of Accell siRNA delivery using Accell delivery medium and added directly to cells without the need for lipid transfection or electroporation, allowing for RNAi in difficult-to-transfect cell types. Can be used with Accell delivery medium, as well as low serum medium. **B)** *PPIB* expression in WTC-hiPSCs was reduced by >90% compared to non-targeting control (NTC) without compromising cell viability or iPSC morphology. Scale bar = 100 µm.

Successful target knockdown in HCT-116 spheroids using Accell siRNA

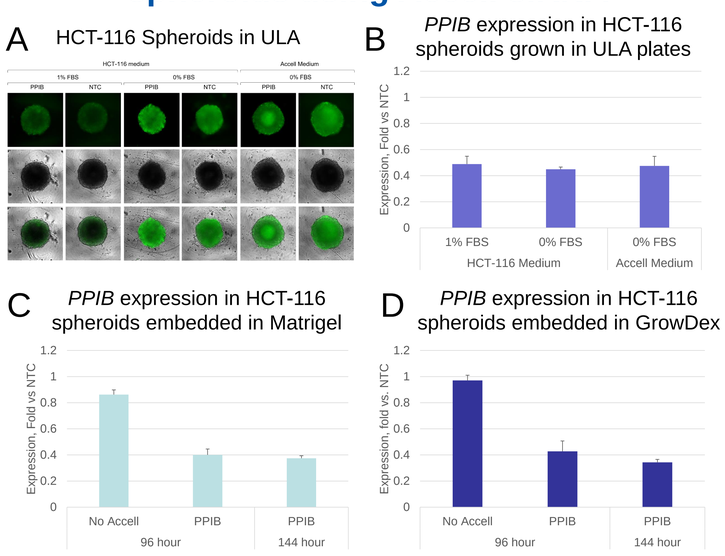


Fig 2. Fluorophore-labelled siRNA reveal siRNA uptake into HCT-116 spheroids, resulting in strong knockdown of target mRNA. **A)** Uptake of fluorophore-labeled Accell siRNA in HCT-116 spheroids formed in ULA plates**. B)** Low serum HCT-116 medium and Accell medium both result in knockdown of PPIB in ULA spheroids using Accell siRNA. **C)** In spheroids formed in Matrigel, long term Accell siRNA treatment in Accell medium resulted in PPIB knockdown. **D)** Spheroids grown in GrowDex also show strong knockdown after treatment with Accell siRNA. *NTC = Non-Targeting Control*

Successful target knockdown in LS174T spheroids using Accell siRNA

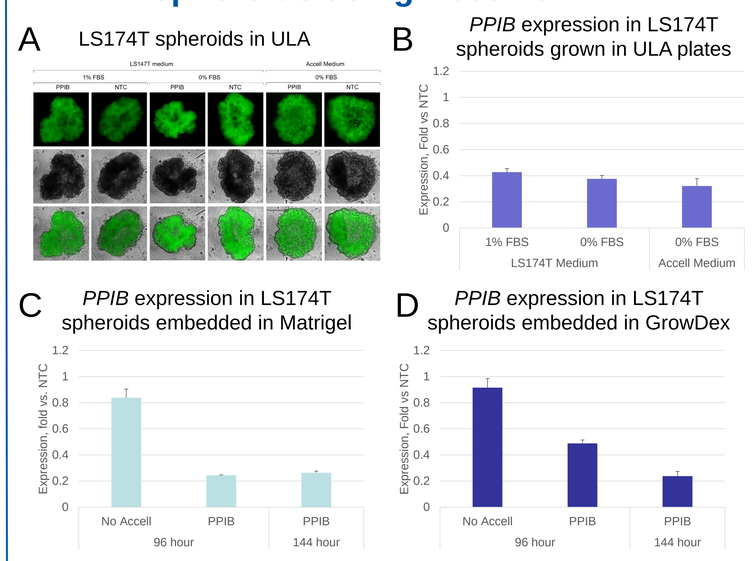


Fig 3. Fluorophore-labelled siRNA reveal siRNA uptake into LS174T spheroids, resulting in strong knockdown of target mRNA. **A)** Uptake of fluorophore-labeled Accell siRNA in LS174T spheroids formed in ULA plates. **B)** Low serum LS174T medium and Accell medium both result in knockdown of PPIB in ULA spheroids using Accell siRNA. **C)** In spheroids formed in Matrigel, long term Accell siRNA treatment in Accell medium resulted in PPIB knockdown. **D)** Spheroids grown in GrowDex also show strong knockdown after treatment with Accell siRNA. *NTC = Non-Targeting Control*

Multiplexing Accell siRNA in HCT-116 spheroids formed in ULA plates result in specific target mRNA knockdown despite sequence homology

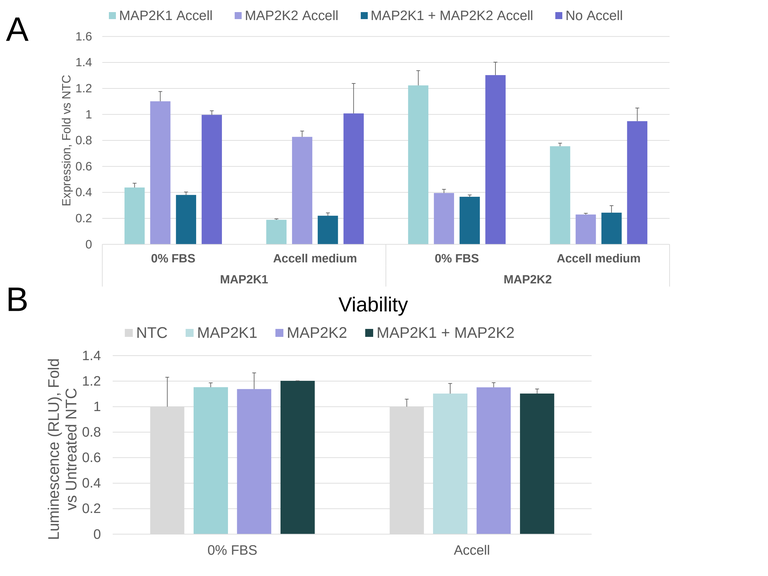
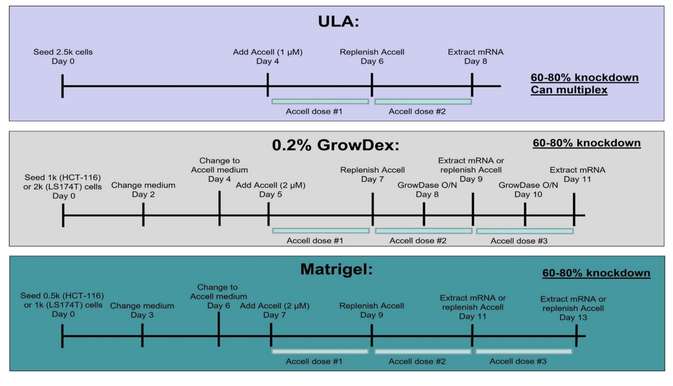


Fig 4. Targeting the *MAP2K1* and *MAP2K2* genes simultaneously with Accell siRNA. A) Successful mRNA knockdown 96h post transfection when multiplexing Accell siRNA treatment targeting *MAP2K1* and *MAP2K2* genes with high specificity despite sequence homology **B)** No effect on viability of cells 96h post transfection.

Experimental designs using Accell siRNA in three different spheroid models



Conclusion

Here, we highlighted the successful use of Accell siRNA in three models of generating spheroids. Using Accell siRNA in combination of GrowDex resulted in the strongest knockdown of the target gene in spheroids generated either with the HCT-116 or the LS174 cell line. Multiplexing two Accell siRNA result in equal efficient knockdown compared to a single Accell siRNA treatment. Combined with our extensive experience with synthetic RNA, these tools enable the interrogation of genetic mechanisms in spheroids and hiPSC using RNAi.